Helicobacter pylori Eradication Using Laser Endoscope and Methylene Blue

Kouji Ogasawara [1, 2], Susumu Nakajima [3], Hiroshi Sato [4], Tadashi Sasaki [4]

1: Sarufutsu Village National Health Insurance Hospital, Japan
2: Asahikawa Medical University, Respiratory Center, Japan
3: Moriyama Memorial Hospital, Japan
4: Sarufutsu Village National Health Insurance Hospital

Background and Aims: *Helicobacter pylori* (*H. pylori*) eradication has become increasingly unsuccessful due to the prevalence of antibiotic resistance. To address this global issue, a novel strategy for eradication without antibiotics must be developed. The purpose of this study was to examine the effect of methylene blue (MB) with sodium bicarbonate ($NaHCO_3$) on *H. pylori* using photodynamic antimicrobial chemotherapy.
Materials and Methods: MB was basified using $NaHCO_3$. The basic effect of MB with $NaHCO_3$ was examined using an endoscope equipped with a laser light source. *H. pylori* was smeared on the culture media with basic MB, followed by illumination at approximately 1,100 lux for 10 and 20 seconds.
After 4 days of culture, the basic effects were determined according to the bacterial growth.
Results: The basic effects of MB appeared at a pH from 8.6 to 9.0 and at $NaHCO_3$ concentrations between 2% and 6.5%. MB concentrations of > 0.05% exhibited the basic effects. The duration of irradiation had no remarkable effects.
Conclusions: Our results showed that the laser endoscope and basic MB were effective for *H. pylori* eradication.

Key words: *Helicobacter pylori* · *methylene blue* · *sodium bicarbonate* · *basic effect* · *laser endoscope*

Introduction

The bacteria *Helicobacter pylori* (*H. pylori*) are gram-negative, flagellated, microaerophilic, and spiral or curved bacilli present in almost all patients with active chronic gastritis, duodenal ulcers and gastric ulcers [1)] and *H. pylori* infects half of the worldwide population and plays a causal role in ulcer diseases and gastric cancer [2)].

For eradication, a combination therapy of proton pump inhibitors and antibiotics has been used. However, because of the development of widespread antibiotic resistance, complete eradication is difficult to achieve.

Although the development of antibiotic-independent methods has garnered much attention, it yet has to be established.

One study on the motility of *H. pylori* in gastric mucin with an acidic or neutral pH and in the absence of urea showed that the bacteria swam freely at a high pH but were strongly constrained at a low pH [3)].

To infect the stomach and establish colonies on the mucosal epithelial surface, the bacterium has to move across the gel-like gastric mucus lining under acidic conditions [4)].

An acid–base disturbance regulated by an external pH appears to destroy the survival mechanisms of *H. pylori*.

Photodynamic antimicrobial chemotherapy (PACT), which combines light and a photosensitizing drug, is a potential therapy that promotes a phototoxic effect on the targeted cells by oxidative damage [5)].

We developed a basic pH condition by adding sodium bicarbonate ($NaHCO_3$) to methylene blue (MB) and, consequently, devised a new photochemical method using basic MB. We performed PACT and aimed to verify its

Addressee for Correspondence
Kouji Ogasawara
Director of Sarufutsu Village National Health Insurance Hospital, Hokkaido, Japan
Asahikawa Medical University, Respiratory Center, Japan
Phone No: +81-1635-2-3331 Fax No: +81-1635-2-3500
E-mail: koujioga@topaz.ocn.ne.jp

Received date: November 6th, 2019
Accepted date: February 19th, 2020
J-STAGE Advance Publication Date: April 18th, 2020

bactericidal effect on *H. pylori* using laser endoscope and basic MB.

Materials and Methods

Materials and preparation

We used *H. pylori* strain (Japan Collection of Microorganisms: No.12093) obtained from the National Research and Development Agency, Institute of Physical and Chemical Research (RIKEN, Japan). *H. pylori* was identified using the API Campy kit (bioMérieux Japan Ltd., Tokyo, Japan).

The *H. pylori* colonies were placed in a sterile physiological solution for an adjustment of the count to 2.0×10^8 cells/mL and were stored in a sterile test tube.

We used a 500 mL solution containing 25 g of MB diluted in ethanol (FUJIFILM Wako Pure Chemical Corporation, Japan) as the stock mixture and $NaHCO_3$ powder (Nichi-Iko Pharmaceutical Co., Ltd., Japan). A series of solutions containing MB concentrations of 0.01%, 0.05%, 0.1%, 0.2%, 0.5%, and 1% in sterile water for injection were prepared in sterile test tubes. $NaHCO_3$ solutions at concentrations of 2%, 3%, 4%, 5%, 6%, and 6.5% were made in a similar manner.

Irradiation experiments using a laser endoscope

Using a sterile pipette tip,100 µL of MB, 20 µL of *H. pylori*, and 10 µL of $NaHCO_3$ were taken out of test tubes and were smeared on a *Helicobacter* agar medium (Nissui Pharmaceutical Co., Ltd., Japan) using a sterile bacteria spreader. The bacterial smear area on the medium was adjusted to a circle with a diameter of approximately 4 cm. $NaHCO_3$ was diluted 13-fold before being added to the media, to obtain final concentrations of 0.15%, 0.23%, 0,30%, 0.38%, 0.46%, and 0.50%.

After being left in the dark for 5 minutes, the bacterial cultures were irradiated vertically from a distance of 4 cm using an endoscope system (EG-L600ZW7 scope, Processor VP-7000 system, and Light source device LL-7000; FUJIFILM Co., Tokyo, Japan) with lasers (LASEREO®). The light intensity was adjustable from a darker level (-4) to a lighter level (+5).

For this experiment, the light intensity applied was adjusted to the standard level of automatic control, which was in the middle range of the light intensities. Endoscopic irradiation was performed at approximately 1,100 lux using white light and linked color imaging (LCI) for 10 and 20 seconds.

LCI is an image-processing method that can emphasize minute color differences in the gastric mucosa by simultaneously irradiating a narrowband of short wavelength light and white light [6]. Compared with white light alone, LCI can make reddish colors appear redder and whitish colors appear whiter, thus enabling an easy detection of the subtle changes in colors [6].

After irradiation, which was defined as both white laser light and LCI, the culture media were placed in jars and were incubated under microaerophilic conditions at 37°C for 4 days.

To maintain microaerophilic and moist conditions in the jars, an oxygen absorber-carbon dioxide generator (DIA-Microaerophilic Pack; LSI Medience Corporation, Tokyo, Japan) and 20 ml of sterile water for injection were used.

Determination of effectiveness

The bactericidal effect was evaluated according to the *H. pylori* growth area, which was categorized into six grades. The response was considered ineffective when 1) there was a bactericidal effect in the absence of irradiation and MB; 2) there was a bactericidal effect from irradiation in the absence of MB; or 3) there was a bactericidal effect from $NaHCO_3$ alone, regardless of application of irradiation.

The blank test included the bacteria and each MB concentration with no irradiation.
(–) indicated ineffectiveness.
Compared with the blank test that used an ineffective concentration of 0.01% MB, scores of (1+), (2+), (3+), and (4+) indicated decrease in the growth area by 20%, 40%, 60%, and 80%, respectively.
A score of (5+) indicated no growth area.

Measurements of pH

The pH of MB, $NaHCO_3$, and basic MB solutions were measured. The stock mixture of 0.01% to 1% MB and 2% to 6.5% $NaHCO_3$ were diluted in sterile water in sterile test tubes. The pH values of the 2000 µL MB solution, 400 µL of sterile physiological saline, and 200 µL of $NaHCO_3$ solution that were prepared in sterile test tubes were measured using the Seven Compact instrument (METTLER TOLEDO International Inc.). The concentration of MB was increased from 0.01% to 1% and that of $NaHCO_3$ was increased from 2% to 6.5%; pH was measured for each sample in the series.

Lux measurement of the laser endoscope

Light intensity at the standard level was measured vertically from a distance of 4 cm, using a luminometer (T10-A, KONICA MINOLTA JAPAN, INC.) in the endoscopic room. Lux measurement was performed after adjusting for a room light intensity of 0 lux.

Results

pH of the materials

Table 1 shows the pH values of the MB, $NaHCO_3$, and basic MB solutions.

As the MB concentration increased from 0.01% to

1%, the pH decreased from 7.435 to 5.890. Moreover, as the concentration of $NaHCO_3$ increased from 2% to 6.5%, the pH decreased from 8.141 to 8.045.

After adding $NaHCO_3$ to MB, the pH increased in the range from 8.550 to 9.0.

Effects of basic MB using white light

Table 2 shows the basic effect of MB with $NaHCO_3$ using white light. MB concentrations > 0.5% showed Grade 5+ bactericidal activity in the absence of light. MB concentrations > 0.1% exhibited Grade 5+ bactericidal effect using white light for 10 and 20 seconds. After adding the 2% to 6.5% $NaHCO_3$ concentrations to MB, Grade 5+ bactericidal effect was shown at MB concentrations > 0.05%. The effectiveness did not differ according to the exposure time.

These effects on the media are shown in **Figures 1 to 3**. The basic effect of MB with $NaHCO_3$ was more efficacious, compared with that of MB alone.

Effects of basic MB using LCI

The effect of MB with $NaHCO_3$ using LCI is shown in **Table 3**. MB concentrations > 0.2% had Grade 5+ bactericidal effect after LCI for 10 and 20 seconds. After adding the 2% to 6.5% $NaHCO_3$ concentrations to MB, Grade 5+ bactericidal effect was achieved at MB concentrations > 0.05%. The duration of irradiation had no effect on the basic effect of Grade 5+.

Figures 4 and 5 show these effects on the media. At MB concentrations of > 0.05%, the basic effect of MB

Table 1: pH of MB According to the $NaHCO_3$ Solutions.

NaHCO3 concentration%	MB concentration%						
	0	0.01	0.05	0.1	0.2	0.5	1
0		7.435	6.997	6.856	6.962	6.505	5.890
2	8.141	8.550	8.574	8.589	8.620	8.708	8.869
3	8.109	8.598	8.612	8.617	8.658	8.748	8.939
4	8.082	8.605	8.619	8.672	8.653	8.782	9.000
5	8.037	8.600	8.617	8.632	8.674	8.785	8.929
6	8.030	8.617	8.624	8.628	8.671	8.777	8.972
6.5	8.045	8.668	8.667	8.701	8.739	8.849	9.000

MB, methylene blue; $NaHCO_3$, sodium bicarbonate

Table 2: Basic Effect of MB with $NaHCO_3$ Irradiated at Approximately 1,100 Lux for 10 and 20 Seconds Using White Light.

NaHCO3 concentration		MB concentration %						
%	irradiation (sec)	0	0.01	0.05	0.1	0.2	0.5	1
0	10	—	(−)	(4+)	(5+)	(5+)	(5+)	(5+)
	20	—	(−)	(4+)	(5+)	(5+)	(5+)	(5+)
2	10	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
3	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
4	10	—	(2+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(2+)	(5+)	(5+)	(5+)	(5+)	(5+)
5	10	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
6	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(3+)	(5+)	(5+)	(5+)	(5+)	(5+)
6.5	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
Blank test (no irradiation)		—	(−)	(−)	(3+)	(4+)	(5+)	(5+)

MB, methylene blue; $NaHCO_3$, sodium bicarbonate

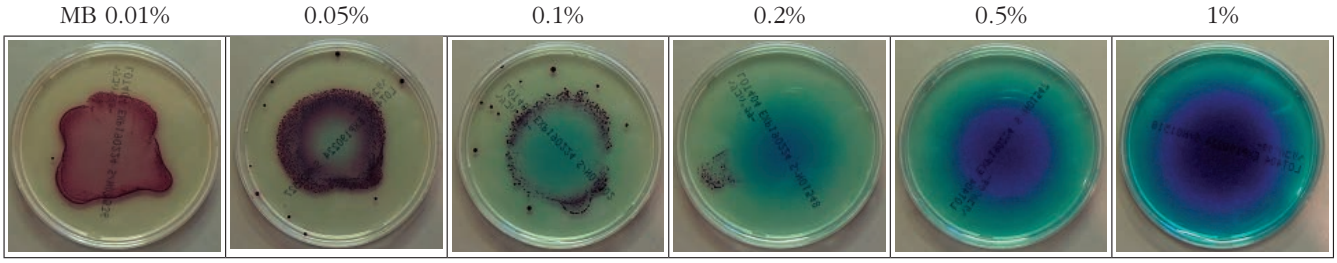

Figure 1: No Irradiation.

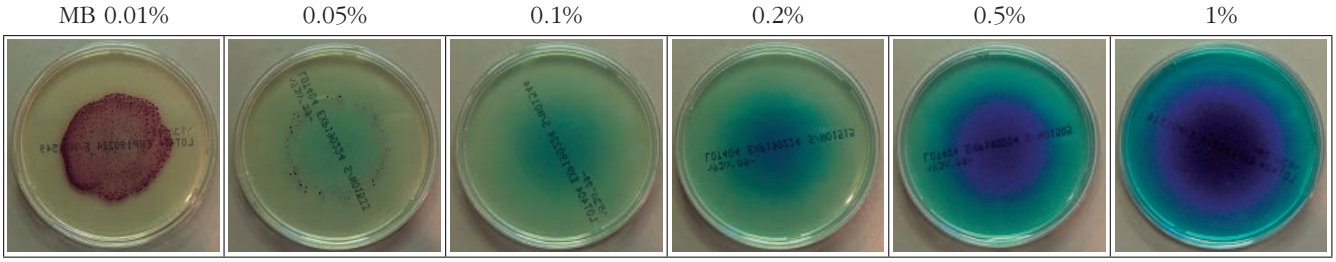

Figure 2: Effect of Methylene Blue Irradiated at Approximately 1,100 Lux for 10 Seconds Using White Light.

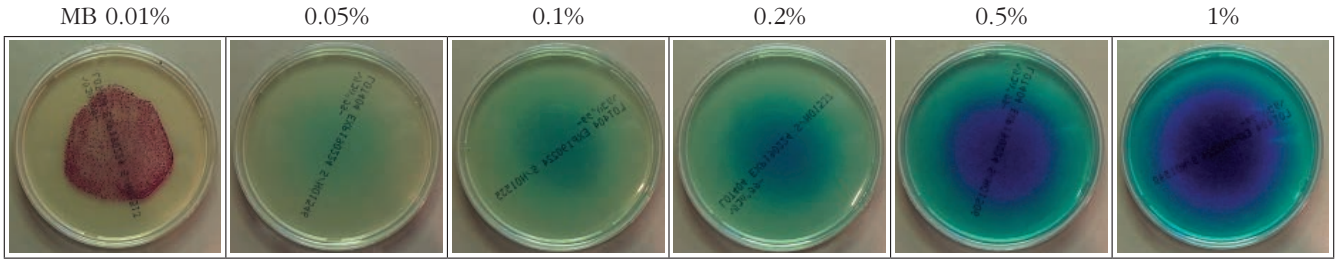

Figure 3: Basic Effect of Methylene Blue with 5% Sodium Bicarbonate Irradiated at Approximately 1,100 Lux for 10 Seconds Using White Light.

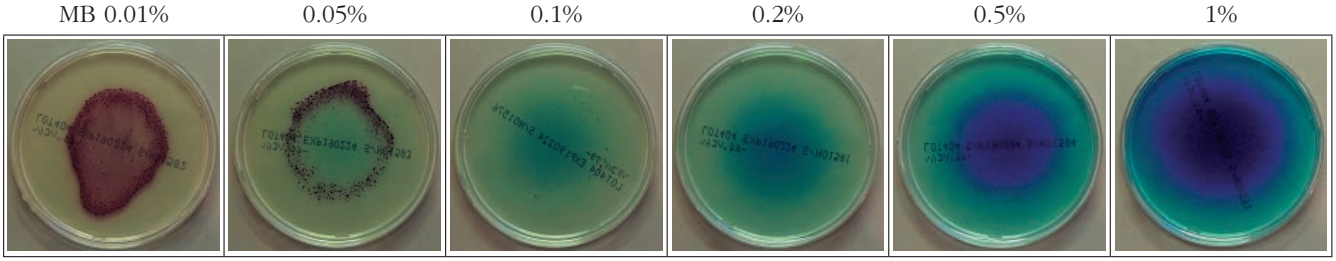

Figure 4: Effect of Methylene Blue Irradiated at Approximately 1,100 Lux for 10 Seconds Using Linked Color Imaging.

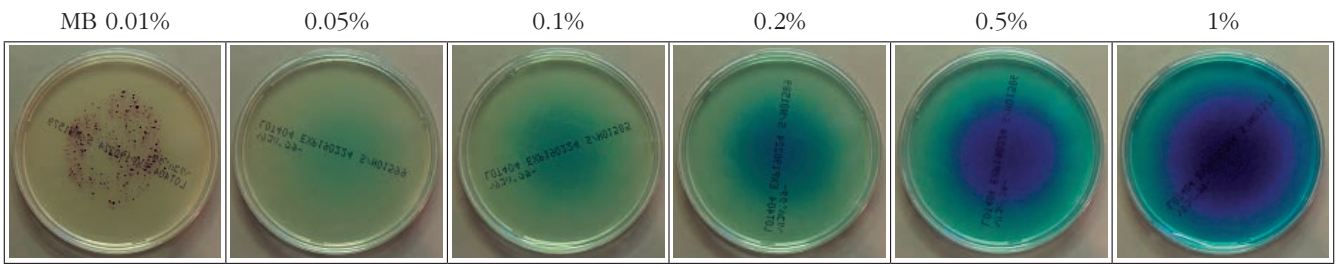

Figure 5: Basic Effect of Methylene Blue with 5% Sodium Bicarbonate Irradiated at Approximately 1,100 lux for 10 Seconds Using Linked Color Imaging.

using white light was consistent with that of LCI for 10 and 20 seconds.

Extent of the basic effect

Figures 6 and 7 demonstrate the relationships among the minimal MB concentration for achieving a Grade 5+ bactericidal effect, $NaHCO_3$ concentration, and pH using white light and LCI.

The colored part of the figures represents the region with extensive basic effect. The basic effects of MB were estimated to be present at a pH between 8.6 and 9.0 and at $NaHCO_3$ concentrations between 2% and 6.5%. The actual $NaHCO_3$ concentrations were in the range of 0.15%–0.50%.

Discussion

In this study, laser endoscopic eradication of *H. pylori* using basic MB with $NaHCO_3$ was highly effective. The combination of acid–base disturbance and PACT is a novel approach to antibiotic-independent eradication of *H. pylori*.

However, this method cannot be applied to patients who do not consent to endoscopy and may not achieve

Table3: Basic Effect of MB with $NaHCO_3$ Irradiated at Approximately 1,100 Lux for 10 and 20 Seconds Using LCI.

NaHCO3 concentration		MB concentration %						
%	irradiation (sec)	0	0.01	0.05	0.1	0.2	0.5	1
0	10	—	(−)	(3+)	(4+)	(5+)	(5+)	(5+)
	20	—	(−)	(3+)	(4+)	(5+)	(5+)	(5+)
2	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
3	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
4	10	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
5	10	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(−)	(5+)	(5+)	(5+)	(5+)	(5+)
6	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(3+)	(5+)	(5+)	(5+)	(5+)	(5+)
6.5	10	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
	20	—	(4+)	(5+)	(5+)	(5+)	(5+)	(5+)
Blank test (no irradiation)		—	(−)	(−)	(3+)	(4+)	(5+)	(5+)

MB, methylene blue; $NaHCO_3$, sodium bicarbonate; LCI, linked color imaging

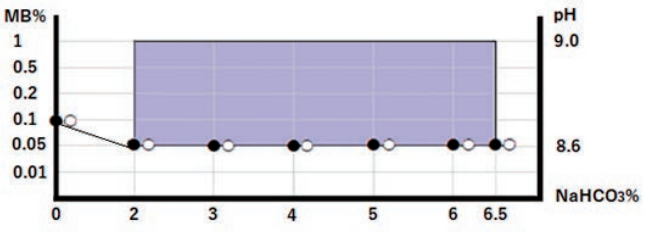

Figure 6: Basic Effect of Methylene Blue with Sodium Bicarbonate Irradiated at Approximately 1,100 Lux for 10 and 20 Seconds Using White Light.

The basic effect is extensively distributed between 2% and 6.5% of sodium bicarbonate ($NaHCO_3$). The black circle (●) and white circle (○) represent the minimal MB concentrations for achieving Grade 5+ bactericidal effects for 10 and 20 seconds, respectively.

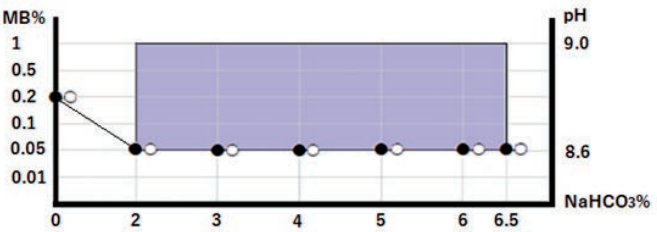

Figure 7: Basic Effect of Methylene Blue with Sodium Bicarbonate Irradiated at Approximately 1,100 Lux for 10 and 20 Seconds Using Linked Color Imaging.

The basic effect is widely present in the range of 2.5% to 6.5% of $NaHCO_3$. The black circle (●) and white circle (○) represent the minimal MB concentrations for achieving Grade 5+ bactericidal effects for 10 and 20 seconds, respectively.

eradication in one session. In such cases, repeated endoscopic eradication is required.

The mechanism of action of the basic effect was considered to involve the properties of PACT as well as the dye used.

General features of dyes

MB, a widely known histological dye [7], was used as a photosensitizer for PACT. Dyes are generally divided into acidic, basic, and amphoteric types [8]. In an aqueous solution, acidic dyes are negatively charged, basic dyes are positively charged, and amphoteric dyes have both features and can maintain an equilibrium state [8]. MB is a water-soluble and basic dye, which has shown affinity to most bacteria and an enhanced staining ability in the basic state [8, 9].

Moreover, MB is a phenothiazine dye that has a strong absorbance in the range of 550–700 nm [10], which is included in the broad wavelength range of endoscopic light. Red light with a wavelength of 660 nm corresponds to the absorption spectrum of MB [11].

Therefore, a laser endoscope can function as a new therapeutic approach for *H. pylori* eradication.

Cell wall and membrane of gram-negative bacteria

Being a microaerophilic gram-negative organism, *H. pylori* has a cell wall, an outer membrane, a periplasmic space, and an inner or cytoplasmic membrane that separates the cytoplasm from the medium [12].

The outer membrane of gram-negative organisms is a phospholipid bilayer that contains a variety of proteins; some exhibit membrane spanning, whereas others are associated with only one face of the bilayer as well as lipopolysaccharides [13].

To exert bactericidal activity, MB has to be extensively distributed within the cell. Notably, MB has no difficulty in crossing bacterial cell walls [14]; because of its cationic charge, it can bind to the negative charge of the lipopolysaccharides of gram-negative bacteria [14]. Moreover, even without light, MB has been reported to show natural antifungal and antibacterial activities [14].

The results of this study implied that the natural antibacterial activity of MB was enhanced through the basic effect and in the presence of a laser light source.

Mechanisms of pH homeostasis

(1) Extent of pH for survival and growth

H. pylori bacteria reside deep in the gastric mucus layer; this gastric *Helicobacter* species has been shown to utilize the mucus pH gradient for precise spatial orientation [15].

After colonizing the human stomach, *H. pylori* can encounter a number of environmental stress inducers; one of the most obvious factors is the drastic fluctuation in stomach pH [16].

Nevertheless, *H. pylori* has evolved to acquire a desirable homeostasis for survival under acid–base conditions. Urease-dependent elevation of local pH to nontoxic levels has been thought to allow *H. pylori* survival in vivo [17].

Survival of *H. pylor*i has been demonstrated at a pH range of 4.0 to 8.0, whereas its protein synthesis occurred at a pH between 6.0 and 8.0 [12]. The major energy source for *H. pylori* is glucose, which is converted into metabolic acids [17]. The pH range for *H. pylori* metabolism was shown to be 3.5–8.6; outside these limits, there was an irreversible loss of metabolic capacity [17].

These survival mechanisms were considered a wide range of acid–base adaptation.

(2) Proton motive force

The proton motive force (PMF) allows aerobic bacteria to survive by functioning as an electrochemical gradient for protons across the plasma membrane [18] and as a means for conversion of the energy of substrate metabolism to ATP synthesis [19]

The PMF can be maintained at a relatively constant pH of 4.0 to 8.0, mostly by an alteration of membrane potential; beyond these pH limits, the PMF was shown to decay irreversibly, which correlated with the survival of organism [18].

Figure 8 depicts this concept of acid–base disturbance and pH homeostasis of *H. pylori*. The regulation of pH homeostasis is critically involved in the *H. pylori* survival and growth.

(3) Effect of pH fluctuation

Generation of pH > 8.6 or < 3.5 exceeds the desirable range for survival, metabolism, protein synthesis, and PMF integrity.

H. pylori encounters basic fluctuation as the pH rises to > 8.6, whereas it falls into acidic fluctuation as the pH declines to < 3.5. Therefore, *H. pylori* eradication is strongly influenced by the pH fluctuations.

Photochemical pathway underlying the basic effect

In this study, the addition of $NaHCO_3$ to MB further increased the basic effect in the presence of light.

It is particularly important that PACT is conducted under basic pH conditions. It is estimated that the basic effect is exerted when both the adjusted $NaHCO_3$ concentration and the basic pH are optimized.

A photochemical pathway underlying the basic effect seemed to have been present and could have altered the role of basic MB from staining to bactericidal therapy.

PACT Mechanisms

The mechanism of PACT results from the interaction of photons of visible light with photosensitizers [20]. Following the absorption of a light photon of specific wave-

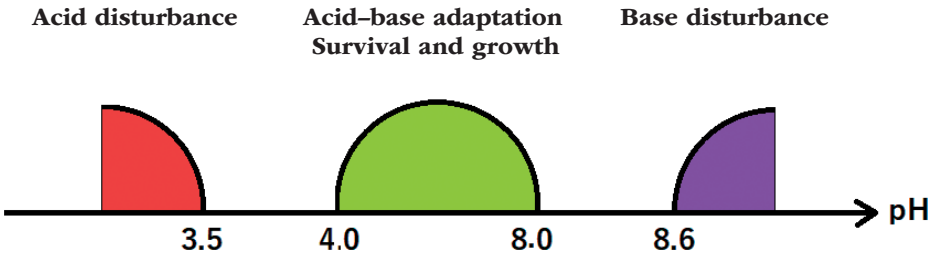

Figure 8: pH Range for the Survival and Metabolism of *H. pylori*.
H. pylori responds to extensive pH fluctuations to maintain survival homeostasis.

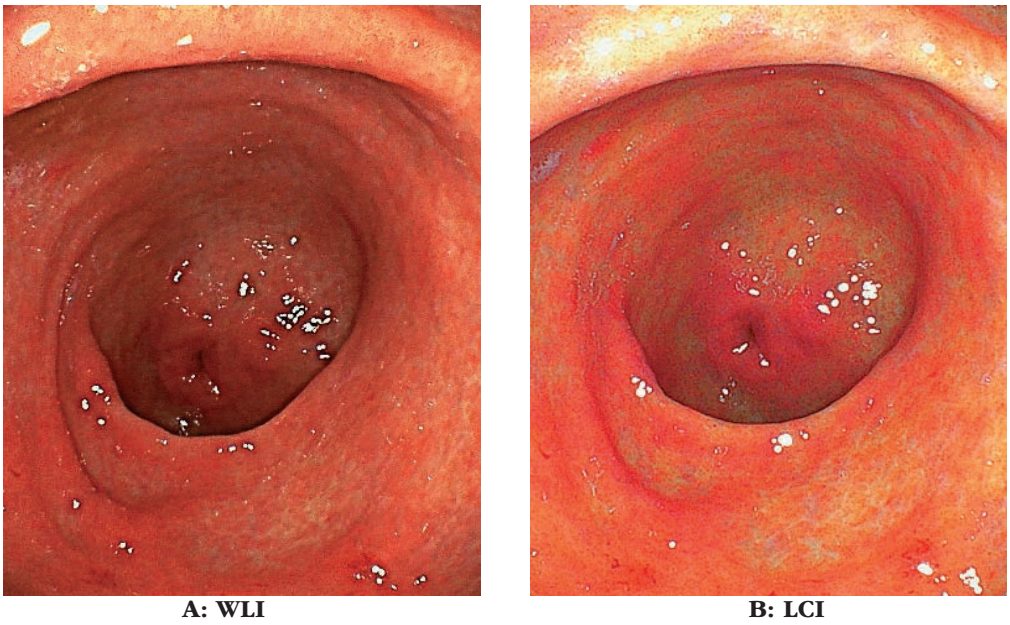

Figure 9: Comparison of the Laser Endoscopic Images of the Gastric Antrum in the Antegrade View. Compared with (A) WLI, (B) LCI highlights the mucosal color changes in atrophic gastritis infected with *H. pylori*.
WLI, white light imaging; LCI, linked color imaging

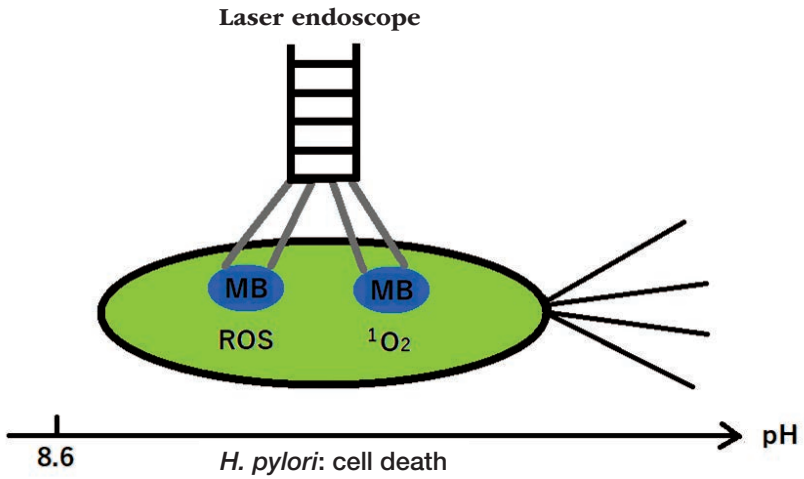

Figure 10: Schema of Laser Endoscopic Eradication of *H. pylori* in the Stomach.

An acid–base disturbance at a pH > 8.6 is a lethal crisis for survival and growth of *H. pylori*. The basic effect of methylene blue (MB) with sodium bicarbonate ($NaHCO_3$) is caused by reactive oxygen species (ROS) and singlet oxygen ($^1O_2$), which are generated through a photochemical activation by endoscopic light. Both ROS and $^1O_2$ induce cell death.

length, the photosensitizer is promoted to a singlet state; thereafter, it is converted to the triplet state, which has a lower energy and longer lifetime relative to the singlet state [20].The excited triplet state of the photosensitizer can generate the production of reactive species, either by Type I or Type II photochemical mechanisms [20, 21, 22]; the former is caused by an electron transfer between the triplet photosensitizer and biomolecules, and it leads to the formation of reactive oxygen species (ROS) [20, 21, 22], whereas the latter is caused by energy transfer to molecular oxygen, which generates singlet oxygen ($^1O_2$) [20, 21, 22]. These cytotoxic ROS and $^1O_2$ induce lethal cell damage.

Mucus dissolution under basic pH conditions

Dissolving the gastric mucus and destroying the external environment are necessary prior to eradication. *H. pylori* uses the gastric mucus pH gradient for chemotactic orientation [15]. The proteolytic enzyme (PRONASE®MS; Kaken Pharmaceutical Co., Ltd. Tokyo, Japan), which is used as a pretreatment for endoscopic examination, is unstable under an acidic state, and exhibits an enzyme activity at a pH ranging from 7 to 10 [23].

This is the reason we cannot eradicate *H. pylori* in an acidic state of pH < 3.5. Therefore, *H. pylori* should be eradicated in a basic state.

Moreover, basic pH conditions can enhance the staining ability of MB and simultaneously optimize the pH of the proteolytic enzyme. At a pH > 8.6, *H. pylori* encounters a lethal pH crisis and is unable to maintain the mechanisms of survival and growth.

Endoscopic eradication using basic MB

As pretreatment during chromoendoscopy, $NaHCO_3$, proteolytic enzyme, and a gastro intestinal antiflatulent agent (GASCON®; Kissei Pharmaceutical Co., Ltd., Tokyo, Japan) [24] are orally administered in the form of aqueous solutions, followed by an endoscopic examination after15 to 30 minutes [25].

When sprayed onto the stomach, MB will not stain normal gastric mucosa, but it will be absorbed by the intestinal epithelium in the presence of intestinal metaplasia [26].

After sufficiently suctioning the dissolved mucus using endoscopic forceps, basic MB is sprayed throughout the gastric mucosa. $NaHCO_3$ is administered twice to achieve a basic pH to optimize the pH of the proteolytic enzyme and to enhance MB staining ability. Exposure to changes in external pH and mucus would disrupt the survival orientation of *H. pylori*.

Distribution of *H. pylori* in the stomach

H. pylori bacteria colonize and typically infect the antral mucosa of the human stomach, but acid suppressive therapy was shown to change the predominance of the infection to the gastric body [27, 28].

After 4 weeks of omeprazole treatment, the histological density of *H. pylori* was reported to decrease in the antrum and corpus, but it increased in the fundus; this migration of *H. pylori* from the antrum to the fundus was associated with a corresponding decrease in the activity of antral gastritis [29]. Moreover, *H. pylori* infection was reported to be a significant risk factor for the development of atrophic gastritis and intestinal metaplasia [30]. Accordingly, *H. pylori* eradication can also suppress the progression of these diseases.

Cmparison of endoscopic images

Figure 9 shows the mucosal color differences in atrophic gastritis between white light imaging (WLI) and LCI. Compared with WLI, LCI was able to emphasize the minute color changes in the gastric mucosa. The WLI function can be easily converted to an LCI function by a manual push button on the top of the endoscope. The use of LCI permitted endoscopic eradication of *H. pylori*, while observing the minute mucosal findings from the infection.

Figure 10 schematically illustrates the mechanisms of laser endoscopic eradication.

Considering the spatial distribution of *H. pylori* in the stomach, laser endoscopic irradiation needs to be extended entirely from the antrum and corpus to the fundus.

A 10-second irradiation on one site of the stomach is conducted multiple times to irradiate the entire stomach.

Stepwise eradication following the generation and maintenance of a basic pH constitutes this photochemical method. PACT holds the potential to improve a successful *H. pylori* eradication.

Conclusions

An acid–base disturbance may be a new therapeutic strategy for *H. pylori* eradication, without producing resistant bacteria. Future human clinical trials including cases of eradication failure are required to verify our results. We believe that laser endoscopic eradication using basic MB can provide a promising technology in clinical practice.

References

1: Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984 Jun 16;1(8390):1311–5.
2: Sachs G, Scott DR, Wen Y: Gastric infection by *Helicobacter pylori*: Curr Gastroenterol Rep. 2011 Dec;13(6):540–6.
3: Celli JP, Turner BS, Afdhal NH, Keates S, Ghiran I, Kelly CP, Ewoldt RH, McKinley GH, So P, Erramilli S, Bansil R: *Helicobacter pylori* moves through mucus by reducing mucin viscoelasticity. Proc Natl Acad Sci USA. 2009 Aug 25;106(34):14321–6.
4: Bansil R, Celli JP, Hardcastle JM, Turner BS: The Influence of Mucus Microstructure and Rheology in *Helicobacter pylori* infection. Front Immunol. 2013 Oct 10; 4:310.
5: Giroldo LM, Felipe MP, de Oliveira MA, Munin E, Alves LP, Costa MS: Photodynamic antimicrobial chemotherapy (PACT) with methylene blue increases membrane permeability in *Candida albicans*. Lasers Med Sci. 2009 Jan;24(1):109–12.
6: Nishida H, Ozawa S, Shimomura K, Abe S, Yoshida K, Ohashi E, Mishima T: Development of a New Endoscopic Platform. FUJIFILM RESEARCH & DEVELOPMENT. 2017; 62:1–7.
7: Tardivo JP, Del Giglio A, de Oliveira CS, Gabrielli DS, Junqueira HC, Tada DB, Severino D, de Fátima Turchiello R, Baptista MS: Methylene blue in photodynamic therapy: From basic mechanisms to clinical applications. Photodiagnosis Photodyn Ther. 2005 Sep;2(3):175–91.
8: Sano Y: Histological Techniques-Theoretical and Applied-. NANZANDO Co., Ltd. Japan. 1981;223–6.
9: Mizuguchi K, Ito K, Shitara M, Higashi K, Hiroi S, Fujita K: The newest complete guide to staining techniques Medical Technology Extra Issue. Ishiyaku Publishers Inc., Japan. 2011;88.
10: Maria C. DeRosa, Robert J. Crutchley: Photosensitized singlet oxygen and its applications. Coordination Chemistry Reviews. 2002 Nov;233–234:351–71.
11: Sousa, João Nilton Lopes de et al: Photoinactivation of *Candida albicans* using methylene blue as photosensitizer. *RGO, Rev. Gaúch. Odontol.* 2015 Dec;63(4):411–7.
12: Scott DR, Weeks D, Hong C, Postius S, Melchers K, Sachs G: The Role of Internal Urease in Acid Resistance of *Helicobacter pylori*. Gastroenterology. 1998 Jan;114(1):58–70.
13: Scott D, Weeks D, Melchers K, Sachs G: The life and death of *Helicobacter pylori*. Gut. 1998 Jul;43(Suppl 1): S56–60.
14: Peloi LS, Soares RR, Biondo CE, Souza VR, Hioka N, Kimura E: Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue. *J. Biosci.* 2008 Jun;33(2):231–7.
15: Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO, Josenhans C, Suerbaum S: The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc Natl Acad Sci. USA. 2004 Apr 6:101(14): 5024-9.
16: Merrell DS, Goodrich ML, Oz tto G, Tompkins LS, Falkow S: pH-Regulated Gene Expression of the Gastric Pathogen *Helicobacter pylori*. Infect Immun. 2003 Jun;71(6):3529–39.
17: Rektorschek M, Weeks D, Sachs G, Melchers K. Influence of pH on Metabolism and Urease Activity of *Helicobacter pylori*. Gsstroenterology. 1998 Sep;115(3):628–41.
18: Meyer-Rosberg K, Scott DR, Rex D, Melchers K, Sachs G: The Effect of Environmental pH on the Proton Motive Force of *Helicobacter pylori*. Gastroenterology. 1996 Oct;111(4):886–900.
19: Sachs G, Meyer-Rosberg K, Scott DR, Melchers K: Acid, protons and *Helicobacter pylori*. Yale J Biol Med. 1996 May-Jun;69(3):301–16.
20: Donnelly RF, McCarron PA, Tunney MF: Antifungal photodynamic therapy. Microbiological Research. 2008;163(1):1–12.
21: Bacellar IO, Pavani C, Sales EM, Itri R, Wainwright M, Baptista MS. Membrane Damage Efficiency of Phenothiazinium Photosensitizers. Photochemistry and Photobiology. 2014 Jul-Aug;90(4):801–3.
22: Gabrielli D, Belisle E, Severino D, Kowaltowski AJ, Baptista MS: Binding, aggregation and photochemical properties of methylene blue in mitochondrial suspensions. Photochemistry and Photobiology. 2004 Mar;79(3):227–32.
23: Pharmaceutical Products Interview Form: PRONASE® MS the third edition. Kaken Pharmaceutical Co., Ltd. Japan. 2010:2–6.
24: Pharmaceutical Products Interview Form: GASCON® the second edition. Kissei Pharmaceutical Co., Ltd. Japan. 2012;8–14.
25: Japan Gastroenterological Endoscopy Society: Gastroenterological Endoscopy Handbook. Japan Medical Center, Japan. 2012:228–9.
26: Kanai M et al: Kanai's Manual Clinical Laboratory Medicine. 32nd edition: Kanehara & Co., Ltd., Japan. 2005:1320–1.
27: Mollenhauer-Rektorschhek M, Hanauer G, Sachs G, Melchers K: Expression of UreI is required for intragastric transit and colonization of gerbil gastric mucosa by *Helicobacter pylori*. Res Microbiol. 2002 Dec; 153(10):659–66.
28: Marcus EA, Sachs G, Scott DR: Eradication of *Helicobacter pylori* Infection. Curr Gastroenterol Rep. 2016 Jul;18(7):33.
29: Logan RP, Walker MM, Misiewicz JJ, Gummett PA, Karim QN, Baron JH: Changes in the intragastric distribution of *Helicobacter pylori* during treatment with omeprazole. Gut. 1995 Jan;36(1):12–6.
30: Kuipers EJ, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG: Long-term sequelae of *Helicobacter pylori* gastritis. Lancet. 1995 Jun 17;345(8964):1525–8.

Acknowledgments
This paper was based on a lecture at "The 29th Annual Meeting of The Japan Photodynamic Association" held on September 19, 2019 in Tokyo.